

**Toxicology Studies on Lewisite and Sulfur Mustard Agents:
Two-Generation Reproduction Study of
Lewisite in Rats**

Final Report

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July 15, 1989

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, MD 21701-5012

Army Project Order No. 84PP4865

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AD-A214 311

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PACIFIC NORTHWEST LABORATORY
operated by
BATTELLE MEMORIAL INSTITUTE
for the
UNITED STATES DEPARTMENT OF ENERGY
under Contract DE-AC06-76RLO 1830

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY ---			3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited		
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE ---			5. MONITORING ORGANIZATION REPORT NUMBER(S) ---		
4. PERFORMING ORGANIZATION REPORT NUMBER(S) PNL-6978			7a. NAME OF MONITORING ORGANIZATION		
6a. NAME OF PERFORMING ORGANIZATION Pacific Northwest Laboratory		6b. OFFICE SYMBOL (If applicable) ---	7b. ADDRESS (City, State, and ZIP Code)		
6c. ADDRESS (City, State, and ZIP Code) PO Box 999 Richland, WA 99352			9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Army Project Order #84PP4865		
8a. NAME OF FUNDING / SPONSORING ORGANIZATION US Army Medical R&D Command		8b. OFFICE SYMBOL (If applicable) ---	10. SOURCE OF FUNDING NUMBERS		
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5012		PROGRAM ELEMENT NO. 63751A	PROJECT NO. 3M2 63751D993	TASK NO. CP	WORK UNIT ACCESSION NO. 003
11. TITLE (Include Security Classification) (U) Toxicity Studies on Lewisite and Sulfur Mustard Agents					
12. PERSONAL AUTHOR(S) L.B. Sasser, J.A. Cushing, D.R. Kalkwarf, P.W. Mellick, and R.L. Buschbom					
13a. TYPE OF REPORT Final		13b. TIME COVERED FROM 9-14-84 TO 7-15-89		14. DATE OF REPORT (Year, Month, Day) 1989 July 15	
				15. PAGE COUNT 43	
16. SUPPLEMENTARY NOTATION Two-Generation Reproduction Study of Lewisite in Rats Appendices available on request from USABRDL as a separate volume.					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP			
24	07		Lewisite, Reproduction, Toxicity, In Vitro Tests, Rats, RA5		
06	11				
19. ABSTRACT (Continue on reverse if necessary and identify by block number)					
<p>Occupational health standards have not been established for Lewisite [bis(2-chlorethyl)-arsine], a potent toxic vesicant which reacts with the sulfhydryl groups of proteins through its arsenic group. The purposes of this study were to determine the reproductive consequences and dose-response of continuing Lewisite exposure of parental males and females and their offspring in a 42-week two-generation study.</p> <p>Solutions of Lewisite were prepared for administration by diluting the neat agent with sesame oil. Rats were administered Lewisite (0, 0.10, 0.25 or 0.60 mg/kg/day for 5 days a week) via intragastric intubation prior to mating, during mating and after mating until the birth of their offspring. The dams continued to receive Lewisite during lactation. At weaning, male and female offspring of each group were selected to continue on the</p>					
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION Unclassified		
22a. NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian			22b. TELEPHONE (Include Area Code) (301) 663-7325		22c. OFFICE SYMBOL SGRD-RM-S

study; receiving Lewisite during adolescence, mating and throughout gestation. Again, the dams continued to receive Lewisite until weaning of the offspring.

Lewisite had no adverse effect on reproduction performance, fertility or reproductive organ weights of male or female rats through two consecutive generations. No adverse effect to offspring were attributed to Lewisite exposure. Minor changes in growth was the only maternal effect observed. Lewisite exposure of parental rats caused no gross or microscopic lesions in testes, epididymis, prostate, seminal vesicles, ovaries, uterus or vagina. Severe inflammation of the lung was observed at necropsy in cases in which Lewisite gained access to the respiratory system from accidental dosing or reflux and aspiration; this usually caused early death of the animal. The NOEL for reproductive effects in this study was greater than 0.60 mg/kg/day.

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EXECUTIVE SUMMARY

Chemical warfare agents present an obvious risk to individuals suffering acute exposure, but they may also present long-term environmental or occupational health hazards for workers in operations involving these chemical agents. Occupational health standards have not been established for Lewisite (L) [dichloro(2-chlorovinyl)arsine] a potent vesicant which reacts with sulfhydryl groups of proteins through its arsenic group. Lewisite is used in a number of research laboratories, stored in depot sites throughout the country and occasionally transported to distant sites. The destruction of current stockpiles of Lewisite by the U.S. Army in the near future could create additional environmental and occupational risk. To establish a database for setting environmental and occupational standards, we conducted studies to evaluate the toxicity, mutagenicity, and reproductive effects of Lewisite using *in vitro* and *in vivo* study systems. The purposes of this multi-generation study were to determine the reproductive consequences and dose-response of continuing chemical exposure of parental males and females and their offspring in a 42 week two-generation study.

Lewisite was administered to three groups of male and female rats prior to mating, during mating, and after mating until birth of the offspring at which time the male rats were sacrificed. The dams continued to receive Lewisite during lactation. At weaning, male and female offspring (F₁) of each group were randomly selected to continue on the study; receiving Lewisite during adolescence, mating, and throughout gestation. Again, the parental males were sacrificed at birth of the offspring and the parental females continued to receive Lewisite until weaning of the F₂ offspring at 3 weeks of age. A fourth group of male and female rats received sesame oil and served as the vehicle controls. Twenty male and 25 female rats were assigned to each of treatment groups and to the vehicle control group for each generation.

Intragastric administration of Lewisite at levels of 0.10, 0.25, and 0.60 mg/kg/day had no adverse effect on reproductive performance, fertility or reproductive organ weights of male and female rats through two consecutive

generations. Likewise, no adverse effects to offspring were attributed to Lewisite exposure. Minor changes in growth was the only maternal effect observed among survivors. For both F₀ and F₁ females, the overall growth curve shows that growth was reduced significantly in the 0.25 mg/kg groups as compared to controls. In addition this response appeared to be stronger for the 0.25 than the 0.60 mg/kg group, but the low number of surviving animals in the 0.60 mg/kg group may have skewed the results. There were no significant changes on reproductive organ weights.

Daily intragastric administration of 0.60 mg/kg of Lewisite to parental rats in the F₀ and F₁ generation caused no gross or microscopic lesions in testes, epididymis, prostate, seminal vesicles, ovaries, uterus, or vagina. No target organ was identified by gross examination of all organs at necropsy or by histologic examination selected tissues. In cases in which the test material gained access to the respiration system by either an accident in dosing or by reflux and aspiration, severe inflammation of lung resulted which usually caused death of the affected animal.

The No-Observable Effect-Level for reproductive effects in this study was greater than 0.60 mg/kg. However, Lewisite was highly toxic and it is doubtful that greater doses could be studied by the intragastric route of exposure because of the sensitivity of the upper respiratory tract and the resulting high maternal mortality.

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INTRODUCTION

Chemical warfare agents present an obvious risk to individuals suffering acute exposure but they may also present long-term environmental or occupational health hazards for workers in operations involving these chemical agents. Lewisite [dichloro(2-chlorovinyl) arsine], one of two major vesicant agents, presents a potential for accidental or occupational exposure because it is used in a number of research laboratories, stored in depot sites throughout the country and occasionally transported to distant sites. In addition, stockpiles of Lewisite are scheduled for destruction by the U.S. Army in the near future, creating an additional potential for environmental and occupational exposure. Although considerable information is known concerning the acute effects of Lewisite, few data are available on its long-term hazards. Segments of the population that may be particularly sensitive to its toxicity include the chronically ill, the young and old, and the unborn. It is this concern that has prompted these studies to identify the potentially toxic, mutagenic and reproductive effects of Lewisite and to establish a database for the development of hazard evaluations and occupational health standards for this chemical.

Lewisite is a highly toxic chemical vesicant. Unlike the strong alkylating vesicant sulfur mustard, Lewisite reacts with the sulfhydryl groups of proteins through its arsenic group (Cassarett and Doull, 1986). In the presence of water or alkalies, Lewisite hydrolyzes to form Lewisite oxide, which is non-volatile and insoluble in water. Although few data are available, Lewisite oxide is generally thought to be a weaker vesicant (Gates et al. 1946) but its toxicity has yet to be comprehensively studied. Relevant chemical and physical data for Lewisite are summarized in Table 1.

A comprehensive review which summarized the chemical and toxicity data of Lewisite acquired during World War I and World War II was published in 1946 (Gates, et al. 1946). This review compared known human and animal data and concluded that sufficient toxicologic data were available for the determination of military usage. Lewisite exposure is characterized by immediate onset of pain, unlike the action of sulfur mustard in which pain may be delayed. The mucus membranes of the respiratory and gastrointestinal

TABLE ' Relevant Chemical and Physical Data for Lewisite,
Dichloro(2-Chlorovinyl)arsine*

Cas #:	541-25-3
RTECS #:	CH2975000
Structural formula:	$\text{Cl}-\text{CH}=\text{CH}-\text{AsCl}_2$
Molecular weight:	207.3 g
Density at 20°C:	1.888 g/ml
State:	Dark, oily liquid (stable in steel and glass)
Vapor pressure at 20°C:	0.394 mm
Decomposition temperature:	>100°C
Solubility in water:	Very slightly soluble
Hydrolysis	
Rate:	Rapid
Products:	Chlorovinyl arsenous oxide, HCl (in acid solutions)
	Acetylene, sodium arsenate (in alkaline solutions)

*Rosenblatt et al. 1975

tracts are particularly sensitive to Lewisite damage. Lewisite is not only a lethal vesicant but is also a systemic toxin; the liver, kidneys, gall bladder, bile duct and other organ systems are vulnerable to damage if absorption occurs (Cameron et al. 1946).

Exposures to Lewisite vapor products edema of the respiratory tract and accumulation of pleural fluid (Gates, et al., 1946). Skin lesions resulting from contact with liquid Lewisite involve the rapid formation of an erythematous area, subsequent vesication and penetration of subcutaneous tissue so that edema and necrosis are evident. Man was less sensitive to skin lesion induction than the dog or rabbit. Systemic intoxication was evident in the dog a few hours following application of Lewisite (Gates et al., 1946). Although sufficient anatomical lesions to characterize the immediate cause of death were not apparent, it was reported that fluid losses due to changes in capillary permeability did cause remarkable decreases in blood volume. Comparisons of toxic effects of Lewisite and sulfur mustard in dogs and rabbits indicated that Lewisite was more damaging to the skin and was more likely to induce systemic poisoning than was sulfur mustard. The

acute LD₅₀ of Lewisite administered via different exposure routes to the rat, rabbit and guinea pig are presented in Table 2.

Table 2. LD₅₀ Values for Lewisite by Various Routes of Administration*

Route of Administration	LD ₅₀ Value (mg/kg)		
	Rat	Rabbit	Guinea Pig
Intravenous	---	0.5, 1.8	---
Subcutaneous	1	2	1
Dermal	15	4	12
Oral	50	---	---

*D.V. Sweet, 1987; Inns, et al. 1988.

Little data are available to evaluate the potential chronic effects of Lewisite other than information based on anecdotal evidence from war use. Based on one incident of accidental exposure to a soldier's leg, Lewisite is considered a suspect carcinogen in man (Krause and Grussendorf, 1978). Workers of a Japanese factory producing mustard and Lewisite agents during World War II had a high mortality rate due to respiratory and gastrointestinal cancers (Wada et al., 1968; Yamakido et al., 1985). These workers were potentially exposed to unknown quantities of both sulfur mustard and Lewisite, therefore it is not possible to implicate Lewisite as a carcinogen because sulfur mustard is a known carcinogen.

Data on the mutagenicity of Lewisite in the literature is limited. Auerbach (1947) found no mutagenic response in the fruit fly exposed to Lewisite and Loveless (1951) reported normal cellular division in root tips exposed to aqueous solutions of Lewisite. Mutagenicity was not detected in recent studies using Ames and Chinese hamster ovary cell assays. (Stewart et al., 1989; Jostes et al., 1989). The teratogenic potential of Lewisite was studied by Hackett et al. (1987) in rats and rabbits using a segment II teratology protocol. Rats were exposed to 0.5, 1.0 or 1.5 mg/kg Lewisite via gastric intubation from 6 to 15 days of gestation (dg) and fetuses were

examined on dg 20. No evidence of a teratogenic response to Lewisite was observed. Likewise, fetal development of the rabbit exposed to 0.07 to 0.6 mg/kg Lewisite between 6 and 19 dg was not affected even though maternal mortality was induced. These results suggest that Lewisite is not teratogenic in the rat or the rabbit after short term exposures since fetal effects were observed only at dose levels that induced maternal toxicity.

It is of interest that many of the symptoms of Lewisite and arsenic intoxication are similar (severe inflammation of the gastrointestinal tract with electrolyte disturbances and ulceration and perforation of membranes) (NAS, 1977) and raises the possibility that the systemic toxicity of Lewisite may result from its arsenic group. In alkaline solutions, Lewisite may hydrolyze to form acetylene and sodium arsenate. Leonard and Lauwerys (1980) reviewed the carcinogenicity, teratogenicity and mutagenicity of a wide variety of arsenic compounds. Some arsenic compounds were mutagenic in bacterial systems while others were not. In general all arsenic compounds tested in mammalian cell systems produced chromosomal aberrations, but no information is available for mutation induction in *in vitro* mammalian systems. Arsenic, as sodium arsenate or arsenite, is known to be embryotoxic and teratogenic in a number of animal species (Leonard and Lauwerys, 1980). In a comparison of Lewisite and sodium arsenite toxicity in the rabbit following intravenous administration, Inns et al. (1988) reported that the LD₅₀ values for sodium arsenite and Lewisite were not similar (7.6 and 1.8 mg/kg, respectively). Furthermore, significant differences in tissue arsenic content and pathology were reported for the two chemicals. Hackett et al. (1987) estimated that the arsenic uptake and accumulation from Lewisite exposure to maternal animals and their fetuses would not be significant at non-lethal doses in short-term teratology studies. However, arsenic accumulation may be important in long-term exposures.

Comprehensive data are not available to evaluate the potential risk to reproduction from long-term occupational exposure to Lewisite. The purposes of this multi-generation study were to determine the reproductive consequences and dose-response of continuing chemical exposure of parental males and females and their offspring in a 42 week two-generation study.

MATERIALS AND METHODS

LEWISITE

Procurement and Characterization

A shipment of 25 ml of dichloro(2-chlorovinyl)arsine (Lewisite, Agent L) was received from the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) on 7 March 1985. The chemical (Lot No. L-U-4273-CTF-N) was prepared by distillation on 30 September 1984 at the Chemical Research and Development Center (CRDC). The agent was analyzed by nuclear magnetic resonance (H-1 and C-13; CRDC SOP No. 8-1-83-1, Annex F) at the Research Directorate, CRDC. Results of the analyses, expressed as calculate weight percent, were 95.8 and 4.0 for trans and cis isomers of dichloro(2-chlorovinyl)arsine, respectively, and 0.2 for unknown compounds.

The Lewisite was divided into two equal portions, pipetted into 30-ml Wheaton vials, sealed and stored in secondary unbreakable containers in refrigerated storage at ~6°C. To comply with Good Laboratory Practices requirements, PNL requested that USAMRICD retain an aliquot of this lot of Lewisite.

Lewisite was analyzed on 20 January 1986 to detect the presence of common impurities, such as Lewisite oxide and the cis-trans isomers of bis(2-chlorovinyl)chloroarsine and tris(2-chlorovinyl)arsine (Rosenblatt et al., 1975). Measurement of the ultraviolet absorption spectrum of the sample in isooctane revealed that the spectrum and the absorptivity of the material at 215 nm agreed with published values in the literature (Rewick, et al., 1986; Mohler and Sorge, 1939) and did not indicate the presence of ultraviolet-absorbing compounds other than Lewisite. This conclusion was supported by results from gas-chromatographic analyses of the sample following derivatization with 2-mercaptoethanol.

Selection and Characterization of Diluent

Lewisite is relatively insoluble and also is rapidly hydrolyzed in water, therefore sesame oil was employed as the diluent for dosing solutions in this study. This selection was not only based on the chemical and

physical properties of the compound, but also on the lack of a toxic response of the vehicle when introduced into the stomach of the animal. Corn oil is commonly the vehicle used for the administration of water-insoluble compounds; however, Hackett et al. (1987) reported from data in the literature that corn oil may not be appropriate for reproductive studies because of its high steroid content and recommended using sesame oil in their studies of the teratology of Lewisite. Sesame oil contains no preservatives, appears to be stable when stored under proper conditions, is relatively low in steroids and is readily available.

The sesame oil (Hain Pure Food Company, Los Angeles, CA) used in this study was purchased locally in one quart bottles and numbered according to lot and bottle. Peroxide analyses of each lot of sesame oil was performed at the beginning of the study or when purchased and periodically throughout the study to provide a measure of oxidation as an indication of rancidity of the oil. The method measures the ability of the oil to oxidize aqueous iodide. Only oil in which the peroxide content was less than 10 meq/kg was used in the study.

The results of the peroxide analyses of the sesame oil used are given in Table 3. The amount of peroxide in the sesame oil was well within the acceptable limits of 10 meq/kg set forth in the protocol.

Preparation of Solutions for Administration

The Lewisite dosing solutions administered to the animals were prepared in advance, approximately every two weeks, and stored in a refrigerator at approximately 6°C. We have previously found that Lewisite is stable in sesame oil for at least 2-3 weeks when stored under these conditions. The general procedure was to determine in advance the amount of neat Lewisite needed, based on the volumes to be prepared and the final concentrations desired. This volume was then removed from the bottle of neat Lewisite and thoroughly mixed into a known volume of sesame oil. Aliquots of this intermediate concentration were then diluted further to give the final concentration needed for the dosing solutions. Aliquots of the final solutions were placed in Wheaton bottles with teflon-lined septa and aluminum caps. Each Wheaton bottle contained sufficient volume of Lewisite-sesame oil

TABLE 3. Analysis of Sesame Oil for Peroxide

Lot No.	Date Purchased	Assay Date	Container Identification	Peroxide meq/kg
51564-6	10/06/86	03/02/87	11	8.1
51565-09	03/04/87	03/13/87	3	6.8
		05/01/87	12	7.9
51566-30	04/10/87	05/01/87	5	2.3
		06/29/87	12	2.6
51566-38	06/16/87	06/29/87	2	0.8
		11/24/87	12	0.7
51566-55	08/31/87	11/24/87	2	3.6
		11/24/87	12	3.5
51566-69	11/13/87	11/24/87	1	0.9

for 1 day's use. The bottles were labeled with the name and the concentration of the agent (Lewisite) and placed into a secondary unbreakable container which was identified by chemical name, concentration, lot number and date prepared.

Analyses of Lewisite Solutions

Lewisite in sesame oil was assayed by gas chromatography, using a capillary column and flame-ionization detection. Substantial analytical problems were encountered during our initial studies (dose-range and teratology studies in rats), and the procedure was subsequently modified for analyses of the solutions used in this study. The presence of high-boiling components in the sesame oil required that the temperature of the capillary-column inlet be maintained at 200°C. Since the decomposition temperature for Lewisite is low (190°C), it was necessary to develop an assay that would permit the migration of sesame oil through the column without any decomposition of the Lewisite. To solve this problem, a stable derivative of

Lewisite in sesame oil was prepared by the addition of 2-mercaptoethanol. The reaction, which proceeds at room temperature, may be written:



In the procedure developed for the analysis, Lewisite samples with concentrations ≤ 2.0 mg/ml were diluted 1:10 with isooctane prior to analysis. For the assay, 0.5 ml of the sample was diluted with 0.5 ml of a solution containing 120 ng of 1-chloronaphthalene and 5584 ng of 2-mercaptoethanol/ μ l in isooctane contained in a 1.5 ml automatic sampler vial with a Teflon-lined, crimped-top cap. The column (J&W Scientific, DB-5) temperature program was 80°C for t min (5°/min) to 140°C, 20°/min to 300°C and 300°C for 20 min. A Hewlett-Packard® 5840A gas chromatograph and a 7672A automatic sample changer were used.

The results of the analyses for Lewisite in the five solutions selected for analysis are shown in Table 4. The results were within acceptable limits of analytical error ($\pm 10\%$) for all five samples of the 0.36 mg/ml concentration and for most concentrations prepared and analyzed in the final phase of the study. The method was not sufficiently sensitive to evaluate the 0.15 or 0.06 mg/ml concentrations during the early phase of the study. The probable cause for the lack of sensitivity at the low concentrations may be interference by some constituents of the sesame oil.

ANIMAL MAINTENANCE

Four week old male and female rats of Sprague-Dawley derivation were obtained from Charles River Laboratories, Inc., Raleigh, NC facility and quarantined in isolation for about 3 weeks until a health evaluation was completed. The Sprague-Dawley rat was selected because it has been used in a number of previous reproductive studies at PNL including gavage studies of Lewisite thereby providing information for dose estimation. During quarantine the rats were group housed, separated by sex, in stainless-steel wire bottom cages placed on automatic flush racks with an automatic watering system.

The environmental conditions specified for the animal rooms were temperatures of 72±3°F, relative humidity of 50±15%, and a lighting cycle of 12 hours on and 12 hours off. Certified Rodent Chow (#5002) was purchased from Purina and drinking water were provided *ad libitum*. Drinking water supplied to the animal rooms was passed through a reverse-osmotic purification unit and containing two particle filters and a carbon filter.

Table 4. Lewisite (L) Concentration of Dosing Solutions Analyzed
Study for Two-Generation Reproduction Study

Date Prepared	Date Analyzed	Dose Level (mg/kg)	L Concentration (mg/ml)	
			Theoretical	Analyzed*
02/19/87	02/23/87	0.60	0.36	0.34 ± 0.02
		0.25	0.15	0.076 ± 0.03
		0.01	0.06	0.019 ± 0.001
	03/10/87	0.60	0.36	0.310 ± 0.06
04/15/87	04/22/87	0.60	0.36	0.38 ± 0.02
		0.25	0.15	0.041 ± 0.0
		0.01	0.06	0.003 ± 0.0005
07/08/87	07/08/87	0.60	0.36	0.35 ± 0.007
		0.25	0.15	0.15 ± 0.002
		0.01	0.06	0.04 ± 0.001
11/03/87	11/06/87	0.60	0.36	0.35 ± 0.01
		0.25	0.15	0.072 ± 0.006
		0.01	0.06	0.052 ± 0.005
11/17/87	11/18/87	0.60	0.36	0.35 ± 0.01
		0.25	0.15	0.150 ± 0.002
		0.01	0.06	0.057 ± 0.002

* Mean ± SE

Near the end of quarantine, 10 rats were subjected to a health evaluation and tested for antibodies to viral pathogens. No significant pathogens or lesions were found.

Following isolation the rats were weighed and assigned to the appropriate treatment groups by sex and weight by means of a formal randomization statistical package (see Statistical Methods). Each animal was assigned an individual identification number by means of a metal ear tag. The animals were individually housed in wire bottom cages on flush racks and cage cards were used to indicate the animal number and treatment group. Prior to parturition (no later than dg 17) and during lactation the females were housed in solid bottom littering cages (1 litter per cage) utilizing hardwood chip bedding.

Experimental Design

The experimental design for the two-generation reproduction study is outlined in Figure 1. Lewisite was administered to three groups of male and female rats prior to mating, during mating, and after mating until birth of

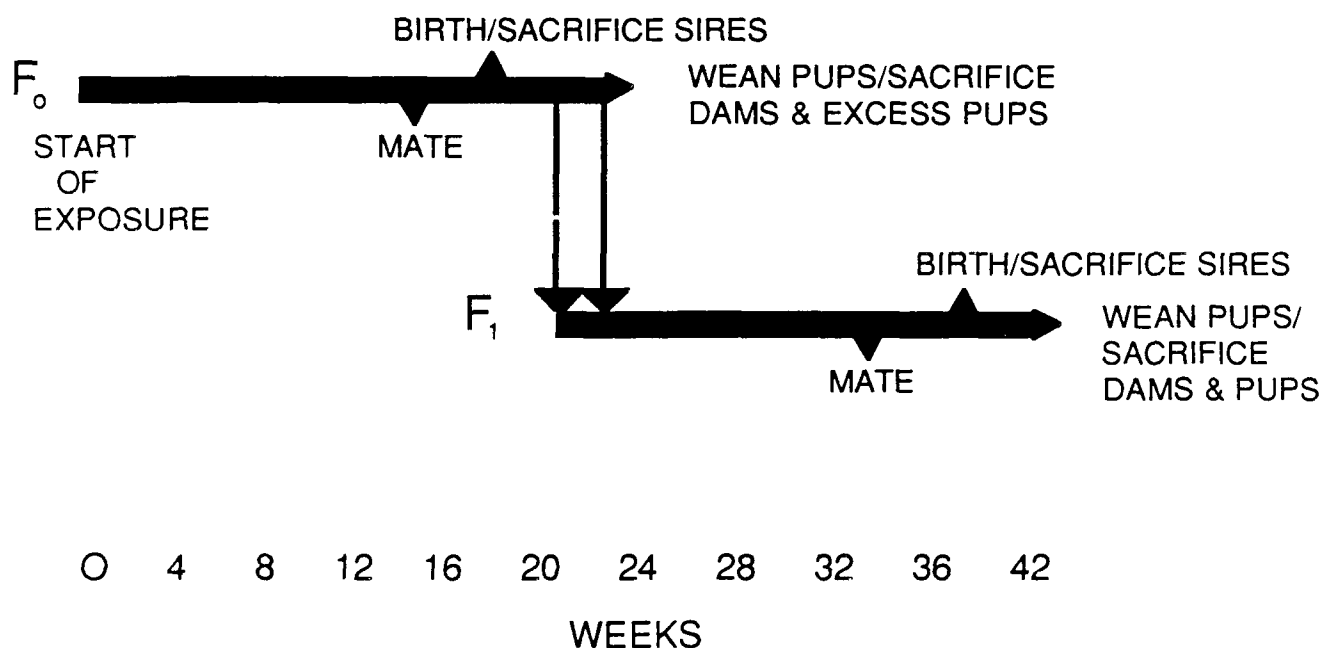


Figure 1. Experimental Design

the offspring at which time the male rats were sacrificed. The dams continued to receive Lewisite during lactation. At weaning, male and female offspring (F_1) of each group were randomly selected to continue on the study; receiving Lewisite during adolescence, mating, and throughout gestation. Again, the parental males were sacrificed at birth of the F_2 offspring and the parental females continued to receive Lewisite until weaning of the offspring at 3 weeks of age. A fourth group of male and female rats received sesame oil and served as the vehicle controls. Twenty male and 25 female rats were assigned to each of treatment groups and to the vehicle control group for each generation (Table 5).

TABLE 5. Treatment groups of the Lewisite (L) Two-Generation Reproduction Study

	Number of Males	Number of Females	Number of Dose Levels	Total Animals
F_0 Generation				
L Exposure Groups	20	25	3	135
Vehicle Control	20	25	1	45
Health Screen	--	--	-	11
F_1 Generation				
L Exposure Groups	20	25	3	135
Vehicle Control	20	25	1	45

Administration of Lewisite

Solutions of the appropriate concentration of sulfur mustard in sesame oil were administered to animals by intragastric intubation, 5 days per week for 13 weeks, until the beginning of the mating period. During gestation, pregnant female rats were dosed 7 days per week. Except for pregnant rats, animals were not dosed on holidays unless a minimum of 4 doses per week could not otherwise be achieved. Dose levels were calculated weekly based on the animal weight, except during pregnancy when the dose was based on the body weight at day 0 of

gestation. Doses were administered in a constant volume of 1.67 ml/kg of body weight. Vehicle control animals were given an appropriate volume of sesame oil.

Dose levels selected for this study were based on data obtained from a dose-range study in pregnant rats, a 3-week rat teratology study and a 90-day subchronic study in male and female rats. A dose level of 1.5 mg/kg did not induce toxic or teratogenic responses in pregnant rats dosed for 10 days beginning on day 6 of gestation. Maternal mortality, decreased maternal and fetal body weights and a reduction in the number of viable fetuses were observed at a dose of 2 mg/kg, but no evidence of teratogenicity was observed (Hackett et al. 1987). In the 90-day subchronic study dose levels of 2.0, 1.0 and 0.5 mg/kg resulted in significant mortality in both sexes and produced lesions in the forestomach. Since it was desired to select doses such that the highest dose induced toxicity but not mortality in the F_0 animals, the low dose not produced any evidence of toxicity, and intermediate dose produce minimal observable toxic effects, dose levels were set at 0.60, 0.25 and 0.10 mg/kg.

Mating Procedures

Breeding of the F_0 and F_1 adult females commenced after each generation had been gavaged with Lewisite for 13 weeks. Females were randomly matched with a male rat of the same dose group for one week; those females which did not mate during the first breeding week were reassigned to a second male and cohabited a second week. The remaining "non-pregnant" females were mated with proven males during a third breeding week. During the 21-day breeding period each female was transferred to the male cage in the late afternoon and was removed each morning and examined for the presence of sperm plugs or sperm in vaginal smears; the morning on which sperm were found was designated as day 0 of gestation. Females becoming pregnant during the 21-day breeding period were selected for continuation in the study. Females not mating during the breeding period and all males were necropsied after the F_0 or F_1 females study animals had been selected. For F_1 matings, cohabitation of siblings was avoided.

Procedures for Newborn Pups

Pregnant females were checked twice each day beginning on 17 dg. At birth the litters were weighed; pups were counted, sexed and examined for viability and gross abnormalities. The date of parturition was recorded and appearance and behavior of dams and pups were observed daily. On day 4 after delivery, the offspring were weighed and the litters were standardized to four male and four female pups per litter; excess pups were killed. If it were not possible to maintain an equal sex distribution within the litter because of a disproportionate sex distribution, a partial adjustment was made in order to maintain a litter size of 8. Litters of less than 8 were not adjusted. Each pup of the litter was uniquely identified with markings on the paws with India ink. The pups were weighed again when they were 14 and 21 of days old. Pups were weaned at 21 days of age and male and female pups of the F_1 generation were randomly selected from each litter for continuation in the study; the excess pups were killed. All F_0 and F_1 adult females and the F_2 pups were killed at weaning.

Twenty male and 25 female pups within each treatment group were randomly selected from the F_0 offspring for the F_1 study. Each F_0 litter was represented by at least one male and one female unless there was a void of either sex within a litter.

Necropsy and Histological Evaluations

A complete gross necropsy was performed on all rats found dead or in moribund condition and those killed at the scheduled sacrifice. Live animals were fasted overnight, euthanitized with 70% CO_2 within one day of the last two consecutive dosings with HD and immediately necropsied. Weights of the testis, epididymis, ovary and uterus were recorded. The lungs were fixed by inserting a blunted needle into the laryngeal lumen through which the fixative was infused. The major organs were stored in 10% neutral buffered formalin (NBF) except for the testes which were fixed Bouin's solution and subsequently washed in 70% ethyl alcohol.

Histopathological evaluations were performed on reproductive organs of the high dose group and control group of the F_0 and F_1 adults. Tissues evaluated

included vagina, uterus, ovaries, testes, seminal vesicles, prostate and epididymides.

STATISTICAL METHODS

The computer software program (DRANDBLK) for randomizing animals into experimental groups was developed by PNL and is based on a single blocking factor for animal weight. Animal weights for a given study were ordered from lightest to heaviest; blocks of animal weights were then randomly assigned to the treatment groups and the control group. Block sizes were governed by the number of test groups.

Analysis of variance was used to analyze body weight, organ weights and forestomach lesion data. When the results of the analyses were significant, Tukey's Studentized Range Test was used to delineate intergroup differences among means (Tukey, 1953). A comparisonwise error rate was set at 0.05 for Tukey's Test. An orthogonal contrast was used to test for a trend in the results repeated over time on the same animal; a randomization test was used to test for differences among growth curves (Zerbe, 1979). This test is a non-parametric statistical test that is based on the absolute area between growth curves and allows for correlation of body-weight measurements over time.

Pairwise comparison of binary response variables between groups was done by chi-square test using the P4F program in the BMDP statistical software (Dixon et al., 1983).

RESULTS

Mortality was high among F₀ and F₁ female rats, especially those exposed to 0.60 mg/kg Lewisite. Two F₀ females (one control and one 0.60 mg/kg) died during parturition probably as a result of breach births. The probable cause of death of three other animals was gavaging errors. The cause of most of the remaining deaths appeared to be associated with aspiration of the test material into the upper respiratory tract as previously described in a 90-subchronic study of Lewisite (Sasser et al., 1989a). This phenomenon appeared to be more severe for the females than for males in this study.

Body weights during the pre-breeding exposure periods for surviving F₀ and F₁ rats are presented in Tables 6 and 7. Individual weekly weights of Lewisite-treated F₀ male and female rats were not significantly different from control animals. However, the overall growth curve of the 0.25 mg/kg F₀ females was significantly reduced ($P < 0.05$) compared to the growth of the control group (Figure 2). Weekly body weights of F₁ male rats exposed to 0.25 mg/kg Lewisite were significantly reduced compared to controls at weeks 11 and 12; weights of female rats exposed to 0.60 mg/kg were reduced at week 6 (Table 7). The overall growth curves of male and female F₁ rats administered 0.25 mg/kg Lewisite was significantly reduced ($P < 0.05$) compared to controls (Figure 3). Although the body weights of the high dose-group was slightly depressed, there were no significant differences in the overall growth curves of treated animals compared to controls (Figure 3); only the 6-week value for females (0.60 mg/kg) was significantly different ($P < 0.05$) from the control group (Table 7).

Breeding performance during the 3-week lavaging period was not adversely affected by exposure to Lewisite for either F₀ or F₁ animals (Table 8). Female fertility (the number of females delivering a litter expressed as a percentage of females placed with a male) and mating index (number of females delivering live litters expressed as a percentage of the females in which matings were detected) of treated groups were consistently greater than control values. A consistent dose-related increase of fertility index was observed for F₀ females; the fertility index of the 0.60 mg/kg group was significantly greater ($P < 0.05$) than controls. The male mating index (the percentage of males mating successfully with at least one female) also tended to be greater for

TABLE 6. Body Weights (g) of F₀ Male and Female Rats Exposed to Lewsite (Mean \pm SE).

Week	Control	0.10 mg/kg	0.25 mg/kg	0.60 mg/kg
MALES				
0	269.4 \pm 3.5	268.4 \pm 3.2	258.2 \pm 7.0	268.1 \pm 3.6
1	322.8 \pm 4.3	321.2 \pm 4.4	314.7 \pm 3.9	320.7 \pm 4.4
2	369.7 \pm 4.9	368.1 \pm 5.5	358.4 \pm 4.5	365.3 \pm 4.4
3	405.5 \pm 5.5	406.3 \pm 6.6	396.2 \pm 5.2	398.0 \pm 5.2
4	431.6 \pm 5.7	437.6 \pm 7.5	425.8 \pm 5.8	432.6 \pm 5.5
5	459.6 \pm 6.6	462.6 \pm 7.5	452.1 \pm 6.1	460.4 \pm 6.6
6	478.6 \pm 7.1	487.6 \pm 8.1	476.7 \pm 6.3	484.8 \pm 6.7
7	500.8 \pm 7.7	511.3 \pm 9.2	501.0 \pm 6.5	506.4 \pm 7.1
8	521.9 \pm 7.3	530.7 \pm 9.5	517.4 \pm 8.0	523.6 \pm 8.1
9	541.3 \pm 8.0	545.7 \pm 10.0	535.3 \pm 8.8	538.8 \pm 7.6
10	560.3 \pm 8.3	564.9 \pm 10.1	553.4 \pm 8.0	558.1 \pm 7.7
11	572.3 \pm 8.4	580.0 \pm 10.0	568.4 \pm 8.0	571.1 \pm 7.8
12	580.0 \pm 8.7	587.0 \pm 9.5	576.6 \pm 8.0	582.3 \pm 8.1
13	585.6 \pm 9.1	587.8 \pm 9.1	579.0 \pm 7.7	582.0 \pm 8.3
FEMALES				
0	182.1 \pm 1.9	180.3 \pm 2.3	179.0 \pm 2.0	182.2 \pm 1.9
1	207.3 \pm 2.4	201.6 \pm 2.7	200.1 \pm 2.3	208.2 \pm 2.8
2	228.0 \pm 2.8	222.2 \pm 3.2	218.3 \pm 2.5	228.7 \pm 3.4
3	247.6 \pm 2.8	241.6 \pm 3.6	236.4 \pm 2.9	247.1 \pm 3.8
4	261.4 \pm 3.3	255.5 \pm 4.5	251.2 \pm 3.4	265.8 \pm 4.0
5	276.8 \pm 3.4	269.6 \pm 4.9	261.5 \pm 3.5	278.2 \pm 3.8
6	279.6 \pm 3.4	273.4 \pm 5.2	271.9 \pm 3.9	288.8 \pm 4.0
7	295.6 \pm 4.0	282.9 \pm 5.3	281.1 \pm 4.3	297.2 \pm 4.5
8	304.3 \pm 4.6	293.1 \pm 5.7	287.3 \pm 4.4	306.5 \pm 4.9
9	312.7 \pm 5.1	299.5 \pm 6.2	294.7 \pm 4.4	313.2 \pm 5.7
10	322.8 \pm 5.2	308.4 \pm 5.8	303.8 \pm 4.6	323.7 \pm 5.4
11	329.5 \pm 5.8	314.7 \pm 6.5	307.9 \pm 4.4	332.8 \pm 7.0
12	330.0 \pm 5.4	319.0 \pm 6.9	308.7 \pm 4.9	334.0 \pm 6.7
13	337.4 \pm 5.4	326.8 \pm 6.8	315.4 \pm 4.5	339.5 \pm 6.7

Table 7. Body Weights (g) of F₁ Male and Female Rats Exposed to Lewisite (Mean \pm SE).

Week	Control	0.10 mg/kg	0.25 mg/kg	0.60 mg/kg
MALES				
0	81.5 \pm 2.9	84.7 \pm 1.9	81.4 \pm 2.0	84.5 \pm 1.8
1	120.6 \pm 6.5	130.7 \pm 5.5	125.3 \pm 5.8	130.4 \pm 6.0
2	181.1 \pm 9.2	184.4 \pm 9.2	186.8 \pm 7.2	185.1 \pm 8.4
3	246.9 \pm 9.4	247.3 \pm 10.0	250.3 \pm 7.6	246.1 \pm 9.8
4	309.2 \pm 9.7	309.0 \pm 10.4	305.5 \pm 7.9	304.1 \pm 10.5
5	368.8 \pm 9.3	365.6 \pm 10.7	357.1 \pm 7.9	355.8 \pm 10.5
6	415.3 \pm 9.2	411.7 \pm 10.4	393.3 \pm 6.6	396.0 \pm 11.2
7	452.9 \pm 9.1	445.0 \pm 9.3	426.9 \pm 6.8	416.2 \pm 23.1
8	492.6 \pm 9.5	479.9 \pm 9.2	457.4 \pm 7.9	466.9 \pm 11.2
9	514.4 \pm 10.4	506.1 \pm 9.2	478.9 \pm 7.3	496.4 \pm 10.7
10	537.0 \pm 10.8	529.9 \pm 9.4	501.8 \pm 7.8	522.3 \pm 10.8
11	563.0 \pm 11.4	551.1 \pm 10.3	521.3 \pm 8.4*	545.3 \pm 10.9
12	583.6 \pm 12.1	571.6 \pm 11.3	539.4 \pm 9.1*	561.1 \pm 12.7
13	590.8 \pm 11.9	580.9 \pm 11.6	543.8 \pm 8.7	555.5 \pm 17.5
FEMALES				
0	77.4 \pm 2.2	80.1 \pm 1.1	76.4 \pm 1.8	78.3 \pm 2.4
1	110.9 \pm 4.8	116.4 \pm 3.7	112.3 \pm 4.4	116.3 \pm 4.1
2	151.2 \pm 5.5	151.5 \pm 5.1	154.3 \pm 4.5	151.7 \pm 4.9
3	185.6 \pm 4.4	183.1 \pm 3.8	182.5 \pm 3.7	183.1 \pm 4.4
4	212.7 \pm 3.3	209.3 \pm 3.9	206.5 \pm 4.5	205.7 \pm 4.7
5	236.1 \pm 3.9	232.0 \pm 4.0	228.5 \pm 3.7	227.9 \pm 4.4
6	256.5 \pm 4.0	249.0 \pm 4.5	243.9 \pm 4.3	234.9 \pm 6.4*
7	270.9 \pm 3.4	263.0 \pm 4.6	254.4 \pm 4.5	257.8 \pm 6.4
8	288.4 \pm 3.7	275.6 \pm 4.9	270.8 \pm 4.7	278.2 \pm 6.5
9	296.6 \pm 4.0	285.1 \pm 4.8	281.2 \pm 4.2	291.0 \pm 6.4
10	306.6 \pm 4.8	291.6 \pm 5.0	288.2 \pm 4.4	301.6 \pm 7.1
11	318.5 \pm 5.5	303.8 \pm 5.1	300.6 \pm 5.4	308.3 \pm 7.9
12	327.8 \pm 5.7	312.1 \pm 5.9	308.5 \pm 5.7	315.0 \pm 8.4
13	336.1 \pm 5.8	316.5 \pm 6.2	306.5 \pm 6.1	317.2 \pm 10.7

*Significantly different from control value by Tukey's Test ($P < 0.05$).

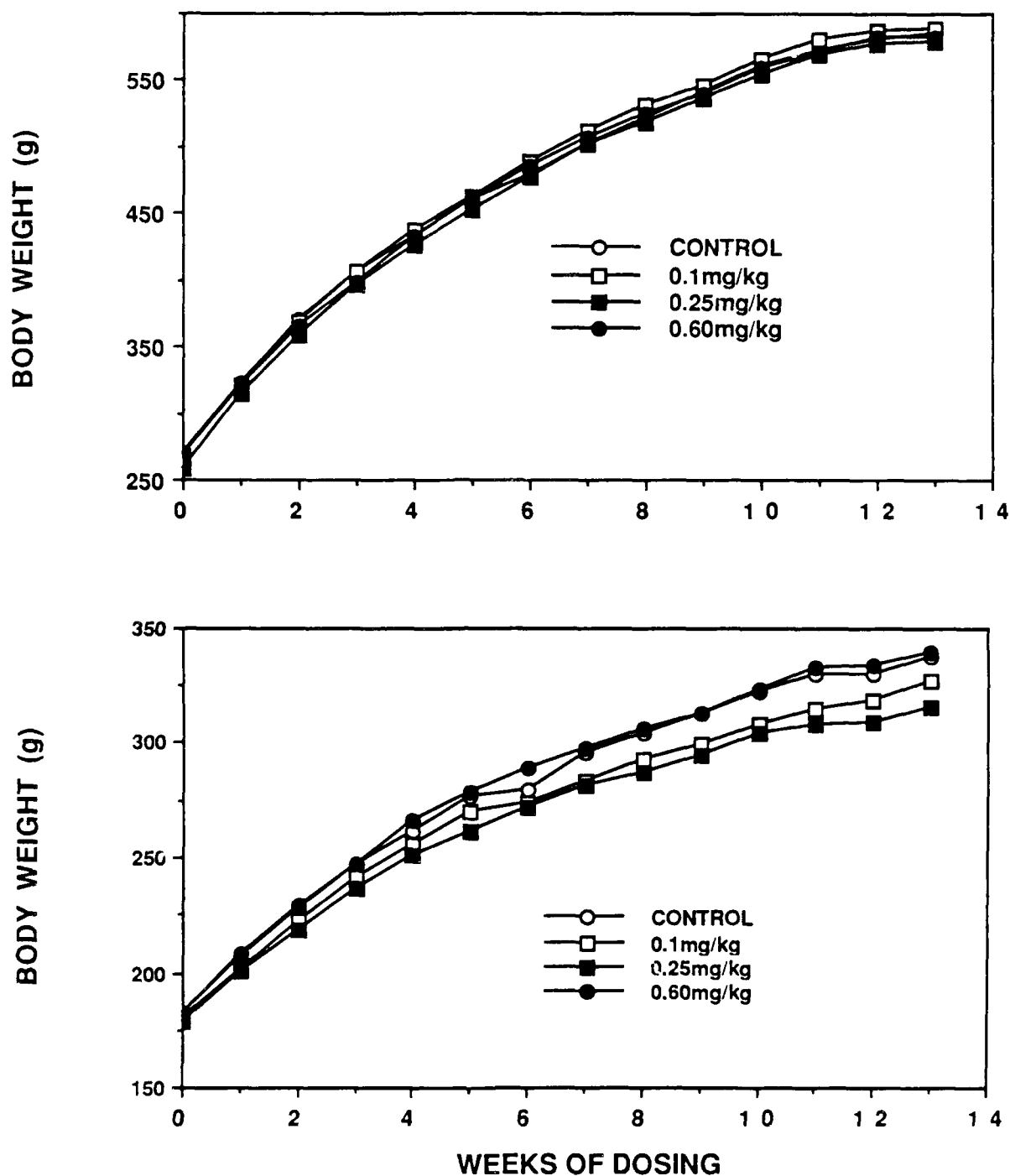


Figure 2. Body weights of F₀ male and female rats gavaged with Lewisite for 13 weeks.

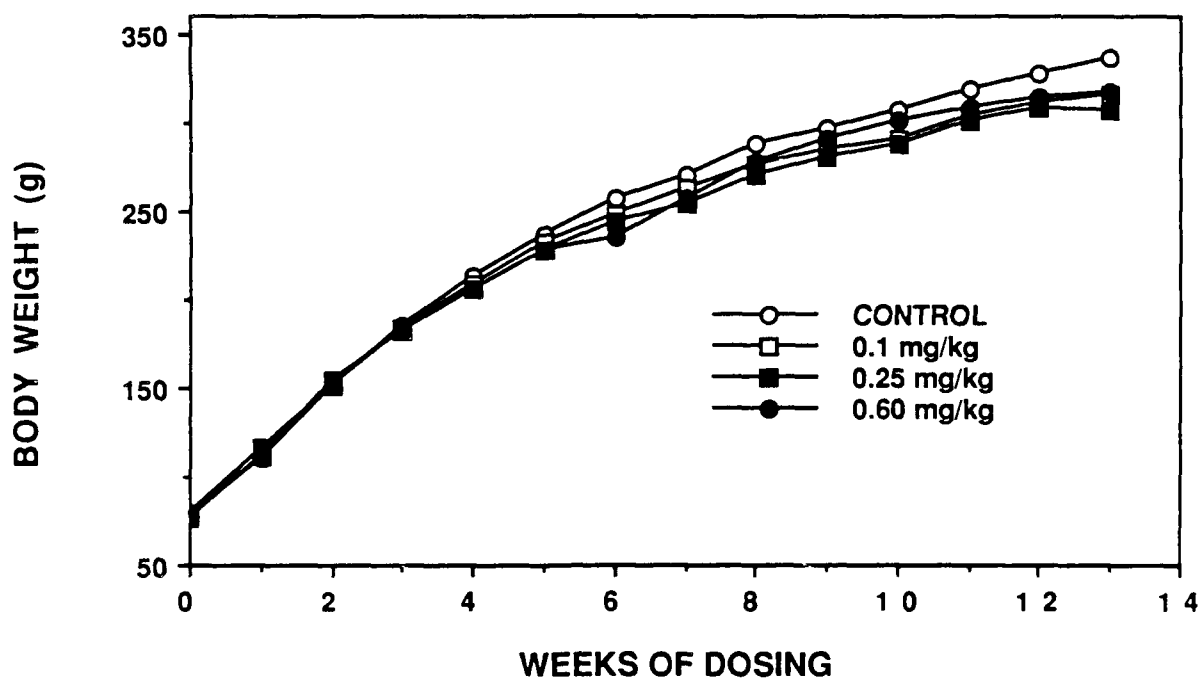
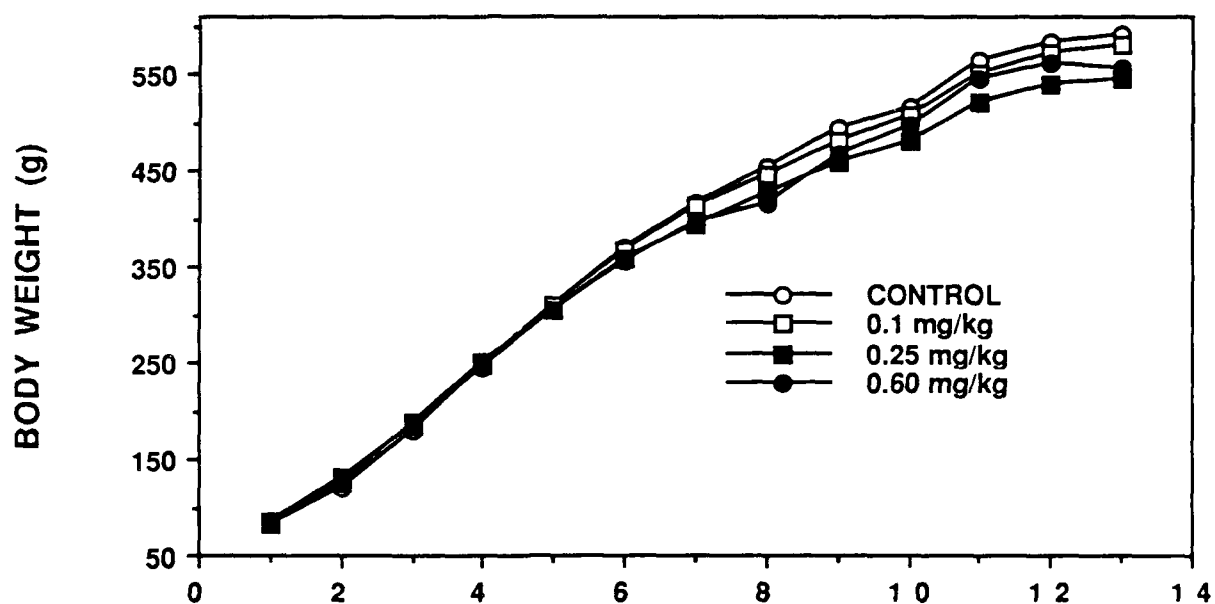


Figure 3. Body weights of F₁ male and female rats gavaged with Lewisite for 13 weeks.

Table 8. Reproductive Performance of F₀ and F₁ Rats Exposed to Lewisite.

	DOSE LEVEL (mg/kg)			
	0	0.10	0.25	0.60
F ₀				
No. Females	25	25	23	19
No. Matings Detected ^a	21	21	22	19
No. Pregnant	14	15	17	17
Pregnant Females Delivering Live Pups (%)	100	100	100	100
Fertility Index (%)				
Female ^b	56.0	60.0	73.9	89.5*
Male	70.0	70.0	95.0	79.0
Mating Index ^c (%)	63.6	71.4	77.3	89.5
F ₁				
No. Females	25	23	22	11
No. Matings Detected ^a	24	21	19	10
No. Pregnant	16	19	10	9
Pregnant Females Delivering Live Pups (%)	87.5	89.5	94.4	88.9
Fertility Index (%)				
Female ^b	64.0	82.6	81.8	81.8
Male	65.0	57.9	50.0	83.3
Mating Index (%)	58.3	80.9	89.5*	80.0

*Significantly different from control (P<0.05).

^aNumber of females in which mating was detected during the 21-day lavaging period.

^bNumber of females delivering a litter expressed as a percentage of females placed with a male.

^cThe number of females delivering live litters expressed as a percentage of the females in which matings were detected.

Lewisite treated animals than for controls, although the only statistically significant ($P < 0.05$) change occurred in F₁ males given Lewisite at a dose of 0.25 mg/kg (Table 8). The biological significance of these positive responses for Lewisite-treated animals is not clear, although the slight Lewisite-induced reduction in body weight may have contributed to improved reproductive performance.

Lewisite treatment did not result in significant changes in litter weights, sex ratio or mean live pup weights for either generation (Table 9). Likewise the number of stillbirths and abnormal pups were unaffected by Lewisite treatment. Only three abnormal pups were found upon gross examination of the litters; two were hairless pups from the same litter (F₀, 0.08 mg/kg) and the other, from the F₀ mid-dose group, had a short or stubbed tail. One F₀ control female failed to deliver her dead fetuses and uterine nidation sites were found in six F₁ females (2 controls, 2 low dose, 2 mid-dose and high dose).

Pup survival through weaning was unaffected by parental exposure to Lewisite. Although pup weights were generally not different among treatment groups, weights at 4 days of age were depressed for F₁ males (0.10 mg/kg) and F₂ females (0.25 mg/kg) (Table 10).

Body weights and weights of selected reproductive organs of F₀ and F₁ males and of F₀ and F₁ females surviving to the scheduled necropsy are presented in Table 11 and 12, respectively. Excluded from these results are data of animals classified as early deaths and of non-gravid females not continued in the reproductive phases of the study. Terminal body weights were generally not adversely influenced by Lewisite treatment, except for a significant decrease ($P < 0.05$) for F₀ females and F₁ males exposed to 0.25 mg/kg Lewisite. Neither absolute nor relative reproductive organ weights of either generation were affected by Lewisite exposure.

Necropsy and Histological Observation

A complete necropsy was performed on all parental animals of the F₀ and F₁ generation. Gross lesions observed at necropsy or tissue trimming were recorded on the Individual Animal Necropsy Record for each animal.

Table 9. Birth Measurements of F_0 and F_1 Exposed to Lewsite
During Pregnancy (Mean \pm SE).

	DOSE LEVELS (mg/kg)			
	0	0.10	0.25	0.60
F_0				
No. Litters	13	14	16	13
Litter Wt. (g)	80.1 \pm 6.5	94.9 \pm 3.6	81.4 \pm 7.5	76.7 \pm 8.52
Sex Ratio (Fraction of Males)	0.51 \pm 0.05	0.45 \pm 0.03	0.57 \pm 0.05	0.50 \pm 0.06
Live Pup Wt. (g/litter)	6.73 \pm 0.16	6.55 \pm 0.14	6.52 \pm 0.32	6.81 \pm 0.32
No. Live Pups	11.7 \pm 0.96	14.5 \pm 0.65	11.8 \pm 1.11	11.1 \pm 1.28
No. Stillbirths per Litter	0.23 \pm 0.12	0.07 \pm 0.07	0.81 \pm 0.43	0.46 \pm 0.39
No. Abnormal Pups	0	2	1	0
Pup Survival Index(%)				
0 to 4-day	100	100	97.2 \pm 1.9	97.8 \pm 2.9
4 to 21-day*	100	99.1 \pm 0.89	100	100
F_1				
No. Litters	13	17	15	5
Litter Wt. (g)	89.3 \pm 2.9	80.2 \pm 6.3	91.4 \pm 4.4	102.0 \pm 4.4
Sex Ratio (Fraction of males)	0.53 \pm 0.05	0.49 \pm 0.03	0.48 \pm 0.03	0.58 \pm 0.08
Live Pup Wt. (g/litter)	6.48 \pm 0.15	6.57 \pm 0.17	6.25 \pm 0.13	6.80 \pm 0.13
No. Live Pups per Litter	13.5 \pm 0.53	12.1 \pm 0.99	14.4 \pm 0.63	15.0 \pm 0.63
No. Stillbirths per Litter	0.38 \pm 0.27	0.41 \pm 0.21	0.20 \pm 0.14	0
No. Abnormal Pups	0	0	0	0
Pup Survival Index(%)				
0 to 4-day	100	97.1	98.2	98.7
4 to 21-day*	99.0 \pm 0.6	100	93.3 \pm 6.7	100

*Survival indices were determined after reducing the number of pups per litter to 8 on day 4.

Table 10. Body Weight (g) of F₁ and F₂ Male and Female Pups During Nursing (Mean \pm SE).

		DOSE LEVEL (mg/kg)			
		0	0.10	0.25	0.60
F ₁					
MALES					
Day	4	12.3 \pm 0.21	11.3 \pm 0.24*	12.2 \pm 0.23	12.3 \pm 0.30
Day	14	38.4 \pm 0.41	38.5 \pm 0.70	39.0 \pm 0.33	38.4 \pm 0.66
Day	21	65.0 \pm 1.04	66.4 \pm 1.16	67.6 \pm 0.58	66.8 \pm 0.93
FEMALES					
Day	4	11.4 \pm 0.21	10.6 \pm 0.22	12.0 \pm 0.22	11.2 \pm 0.32
Day	14	36.8 \pm 0.47	37.6 \pm 0.52	37.8 \pm 0.36	37.3 \pm 0.71
Day	21	62.2 \pm 0.86	63.6 \pm 0.81	65.5 \pm 0.54*	64.0 \pm 1.07
F ₂					
MALES					
Day	4	11.5 \pm 0.21	11.5 \pm 0.23	10.5 \pm 0.25	11.6 \pm 0.24
Day	14	37.8 \pm 0.67	37.6 \pm 0.63	36.8 \pm 0.49	38.4 \pm 0.75
Day	21	63.6 \pm 0.79	63.9 \pm 0.91	62.1 \pm 0.85	63.8 \pm 0.72
FEMALES					
Day	4	11.2 \pm 0.22	11.4 \pm 0.22	10.0 \pm 0.20*	10.9 \pm 0.23
Day	14	37.2 \pm 0.58	37.4 \pm 0.53	35.8 \pm 0.47	36.9 \pm 1.05
Day	21	61.9 \pm 0.77	62.5 \pm 0.79	59.4 \pm 0.78	61.6 \pm 1.08

*Significantly different from control value by Tukey's Test (P<0.05).

Table 11. Body and Reproductive Organ Weights at Scheduled Necropsy of F_0 and F_1 Male Rats Exposed to Lewisite (Mean \pm SE).

Lewisite	N	Body Weight	Epididymis Weight		Prostate Weight		Testes Weight	
mg/kg/day		g	g	mg/100g	mg	mg/100g	g	mg/100g
F_0								
0	20	624.5 ± 11.2	1.29 ± 0.034	207** ± 6	561** ± 34	91 ± 6	3.57 ± 0.11	572 ± 18
0.10	20	620.9 ± 10.5	1.29 ± 0.023	209 ± 5	600 ± 34	97 ± 5	3.56 ± 0.04	576 ± 11
0.25	20	610.8 ± 10.2	1.26 ± 0.030	208 ± 6	528 ± 33	87 ± 6	3.62 ± 0.06	595 ± 12
0.60	16	619.8 ± 10.6	1.28 ± 0.031	206 ± 6	532 ± 38	86 ± 6	3.51 ± 0.06	569 ± 12
F_1								
0	20	626.2 ± 16.8	1.35 ± 0.018	219 ± 6	695 ± 37	112 ± 6	3.73 ± 0.067	603 ± 18
0.10	19	606.9 ± 14.5	1.38 ± 0.021	230 ± 5	634 ± 37	105 ± 6	3.68 ± 0.059	612 ± 14
0.25	18	564.5* ± 9.4	1.29 ± 0.037	229 ± 7	614 ± 33	109 ± 6	3.63 ± 0.059	647 ± 17
0.60	14	622.7 ± 18.0	1.29 ± 0.028	210 ± 8	658 ± 33	107 ± 6	3.65 ± 0.081	591 ± 18

*Significantly different from control value by Tukey's Test ($P < 0.05$).

**N=19

Table 12. Body and Reproductive Organ Weights at Scheduled Necropsy of F₀ and F₁ Female Rats Exposed to Lewisite (Mean \pm SE).

Lewisite mg/kg/day	N	Body Weight g	Uterus Weight		Ovary Weight	
			mg	mg/100g	mg	mg/100g
F ₀						
0	14	360.4 ±7.1	541 ±65	151 ±18	103 ±6	29 ±2
0.10	13	353.8 ±6.5	587 ±61	165 ±17	90 ±4	25 ±1
0.25	16	338.1* ±5.3	562 ±59	168 ±18	99 ±5	29 ±1
0.60	13	367.3 ±7.8	488** ±49	133 ±11	93 ±6	25 ±1
F ₁						
0	13	339.7 ±7.2	552 ±32	163 ±9	116 ±5	34 ±2
0.10	17	322.5 ±5.4	567 ±35	176 ±10	113 ±8	35 ±2
0.25	14	321.1 ±5.3	584 ±56	182 ±17	125 ±6	39 ±2
0.60	3	347.5 ±5.8	610 ±34	176 ±11	132 ±11	38 ±4

*Significantly different from control value by Tukey's Test ($P < 0.05$).

**N=12.

There were no gross lesions directly attributable to the intragastric administration of Lewisite. In the respiratory system of rats that died before scheduled sacrifice, a variety of changes were observed at necropsy. Most of these consisted of fluid accumulation in the thoracic cavity, mottled discoloration of lungs, and small pulmonary foci that were either red or light gray in several animals. In some cases these were probably agonal or early autolytic changes that were not related to administration of the test material. In other animals, the test material entered the respiratory system or thoracic cavity either because of an accident in dosing, or by reflux and aspiration of material administered by gavage. In these cases, the presence of test material in the respiratory system caused a lesion. The incidence and nature of all respiratory changes have been documented in histopathological data.

A number of other gross lesions were observed in control and Lewisite-treated rats in this study. Either these changes did not correlate with treatment and were not considered significant histologically, or there were no corresponding noteworthy microscopic changes.

Protocol-required tissues for histologic examination were ovaries, uterus, vagina, testes, seminal vesicles, prostate, epididymis, and target organs of all control and high dose (0.60 mg/kg) F₀ and F₁ animals selected for mating including those that failed to mate. Target organs identified in the 0.60 mg/kg group were to be examined in lower dose groups as were tissues having gross lesions.

Histopathologic observations were entered directly into the Xybion Path/Tox data management system. The intent was to evaluate microscopically all protocol required tissues. This objective was not achieved since tissues from seven F₀ females in the 0.60 mg/kg group and eight F₀ control females were improperly fixed and unavailable for histologic examination. In addition, one or more tissues from about 20 animals were missing or the available tissues from several animals were unsuitable for evaluation because of autolysis. Therefore, the number of tissues per group examined microscopically was not always the same as the number of animals in the group. Tissues were examined from a total of 179 animals in this study as shown in Table 13.

There were no microscopic lesions attributable to intragastric administration of Lewisite in any of the tissues for which histopathologic examination was required. A variety of non-neoplastic lesions in numerous

Table 13. Histological Examination of F₀ and F₁ rat tissues.

Experimental Group	Number In Group	Early Death	Moribund Sacrifice	Terminal Sacrifice	Not Examined*
F ₀					
Control females	25	1	0	17	7
Control males	20	0	0	20	0
0.60 mg/kg females	25	11	0	7	8
0.60 mg/kg males	20	4	0	16	0
0.25 mg/kg females	25	4	0	0	NA ^a
0.25 mg/kg males	20	0	0	0	NA
0.10 mg/kg females	25	0	0	0	NA
0.10 mg/kg males	20	0	0	0	NA
F ₁					
Control females	25	0	0	25	0
Control males	20	0	0	20	0
0.60 mg/kg females	25	18	1	6	0
0.60 mg/kg males	20	6	0	14	0
0.25 mg/kg females	25	5	0	0	NA
0.25 mg/kg males	20	2	0	0	NA
0.10 mg/kg females	25	2	0	0	NA
0.10 mg/kg males	20	1	0	0	NA

*No histology examination due to improper tissue fixation.

^aNot applicable; no target organs were identified.

organs and tissues, including organs of the reproductive tract, were observed in both control and treated rats. The incidence of these changes was low and not related to experimental treatment with Lewisite. Non-neoplastic changes in the female reproductive tract were mainly associated with pregnancy or parturition. The only histologic lesions in the male reproductive organs were mild changes frequently encountered in rats of this age including mononuclear inflammatory cell infiltration in the prostate in several control and Lewisite-treated animals in both generations, and mild testicular degeneration and oligospermia in the epididymis of one F₀ control male.

Numerous microscopic changes were observed in the lungs of Lewisite-treated rats in both the F₀ and F₁ generations. Since the protocol did not require histopathological examination of the lung unless it had a gross lesion, not all lungs were examined microscopically. Pulmonary lesions included edema, hemorrhage, acute inflammation in both airways and alveoli, subacute inflammation of the pleura and mediastinal tissues, and the presence of foreign material. These lesions were limited to animals treated with Lewisite and all affected animals died prior to scheduled sacrifice. In many instances the pulmonary lesions were severe enough to be considered the cause of death. The lung lesions observed in this study were probably caused by the accidental deposition or reflux of test material into the pharynx and subsequent aspiration of this material into the trachea and lungs. In one F₁ generation male and two F₁ females, there was apparent accidental perforation of the esophagus by the gavage tube and deposition of test material into the mediastinum.

Other microscopic lesions observed in this study were considered incidental findings unrelated to administration of Lewisite and of no consequence in interpretation of results.

DISCUSSION

Results of the present study indicate that exposure, via intragastric intubation, to 0.10, 0.25 and 0.60 mg/kg Lewisite over two generations did not result in significant alterations in the reproductive performance or fertility of the rat. Likewise, no adverse effects to offspring were attributed to Lewisite exposure. Although similar long-term studies have not been conducted, these findings are consistent with those of a short-term teratology study in which no evidence of a teratogenic response was found in rats given up to 1.5 mg/kg Lewisite (Hackett et al., 1987).

The Lewisite dose which may be received by the fetus under these dosing conditions is not clearly known nor can it be evaluated with the limited data available. Degregation of Lewisite is known to occur in aqueous solution, but studies of Lewisite absorption from the gastrointestinal tract or skin and its subsequent metabolism have not been conducted. No data exist regarding the placental transfer of Lewisite. Thus it cannot be positively stated that Lewisite has no adverse reproductive effect until it can be determined whether or not Lewisite is actually transported to the developing fetus.

A majority of the early deaths in this study could be attributed to lesions of the respiratory tract, probably the result of accidental deposition or induced reflux of the test material into the pharynx and subsequent aspiration of the Lewisite into the larynx, trachea, lungs and nasal cavity. These animals did not show signs of Lewisite toxicity, even after long-term exposure, until 2 or 3 days prior to death, suggesting that a single event may have been responsible for the mortality. Evidence of respiratory tract lesions was not found when sulfur mustard, another strong vesicant, was administered at concentrations sufficiently great to cause weight loss and to induce forestomach lesions (Sasser et al., 1989b). Lewisite exposure, unlike the action of sulfur mustard in which pain may be delayed, is characterized by immediate onset of pain; gastrointestinal and respiratory tissues are especially known to be sensitive (Gates et al., 1946). Indeed, Lewisite may have been irritating enough to evoke an immediate attempt by the rat to reject the material causing more accidental dosing trauma or actual aspiration of Lewisite into the respiratory tract than in the case of sulfur mustard. The high mortality

caused by the sensitivity of the respiratory tract prevented the collection of reproduction data at greater doses.

Other than the unique respiratory tract response, a slight reduction in growth was the only maternal effect observed. This response appeared to be stronger for the 0.25 than the 0.60 mg/kg group, but the low number of surviving animals in the 0.60 mg/kg group may have skewed the results.

Daily intragastric administration of 0.60 mg/kg of Lewisite to parental rats in the F₀ and F₁ generations of this two-generation reproductive study caused no gross or microscopic lesions in testes, epididymis, prostate, seminal vesicles, ovaries, uterus, or vagina. No target organ was identified by gross examination of all organs at necropsy or by histologic examination of protocol-required tissues. In cases in which the test material gained access to the respiration system by either an accident in dosing or by reflux and aspiration, severe inflammation of lung resulted which usually caused death of the affected animal.

In summary, intragastric administration of Lewisite at levels of 0.10, 0.25 and 0.60 mg/kg/day had no adverse effect on reproductive performance, fertility or reproductive organs of male and female rats through two consecutive generations. The No-Observable Effect-Level for reproductive effects in this study was greater than 0.60 mg/kg, however, Lewisite was highly toxic and it is doubtful that greater doses could be studied by the intragastric route of exposure because of the sensitivity of the upper respiratory tract and the resulting high maternal mortality.

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STUDY DATES

Animals arrival	02/03/87
Health evaluation	02/20/87
Begin exposure of F ₀ generation	02/20/87
Begin mating F ₀ generation	05/25/87
Begin birthing of F ₁ generation	06/17/87
Begin necropsy of F ₀ excess females	06/15/87
Weaning F ₁ offspring	07/08/87
Begin necropsy of F ₀ females	07/09/87
Begin dosing of F ₁ generation	07/10/87
Begin birthing of F ₂ generation	11/03/87
Begin necropsy of F ₁ males and excess females	11/11/87
Begin sacrifice of F ₂ generation	11/24/87
Begin necropsy of F ₁ females	12/10/87

Data are property of U.S. Army and will be archived under the Army's direction at approved facilities.

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7-31-89
Date

Two-Generation Reproductive Study of Lewisite in Rats

Quality Assurance Statement

Listed below are the phases and/or procedures included in the study described in this report which were reviewed by the Quality Assurance Unit during the period, 1/1/87 - 1/1/88, or specifically for this study and the dates the reviews were performed and findings reported to management. (Findings were reported to the study director or his designee at the time of the review.)

Phase/Procedure Reviewed	Review Date	Date Findings Submitted in Writing to Study Director/Management
Dose Preparation	1/30/87	2/11/87
Animal Identification	2/19&20/87*	3/10/87
Body Weights	2/19&20/87*	3/10/87
Vehicle Analysis	3/2/87*	3/4/87
Clinical Observations	4/6/87*	4/7/87
Dosing	4/6/87*	4/7/87
Body Weights	4/6/87*	4/7/87
Necropsy	5/22/87	5/27/87
Necropsy	7/9&10/87*	7/14/87
Body Weights	7/30/87	8/3/87
Dosing	9/4/87*	9/22/87
Necropsy	11/12/87*	11/12/87
Data	11/12&13/87*	11/13/87
Necropsy	12/10/87*	1/1/88
Data	1/12/88*	1/18/88
Data	1/14/88*	2/1/88
Final Report	9/6,7&22/89	9/22/89

* Reviewed specifically for this study.



 Quality Assurance Auditor

9/22/89

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